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(54) **CURCUMIN-ER, A LIPOSOMAL-PLGA SUSTAINED RELEASE NANOCURCUMIN FOR MINIMIZING QT PROLONGATION FOR CANCER THERAPY**

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See application file for complete search history.

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(57) **ABSTRACT**

The present invention includes compositions and methods of making a nanoparticle composition comprising a polymeric core comprising one or more polymers and one or more active agents, and at least one layer of one or more lipids on the surface of the polymeric core; more specifically, the invention relates to the use of curcumin within such a lipid-polymer nanoparticle formulation for minimizing QT prolongation associated with curcumin in treatment of cancer.

14 Claims, 17 Drawing Sheets

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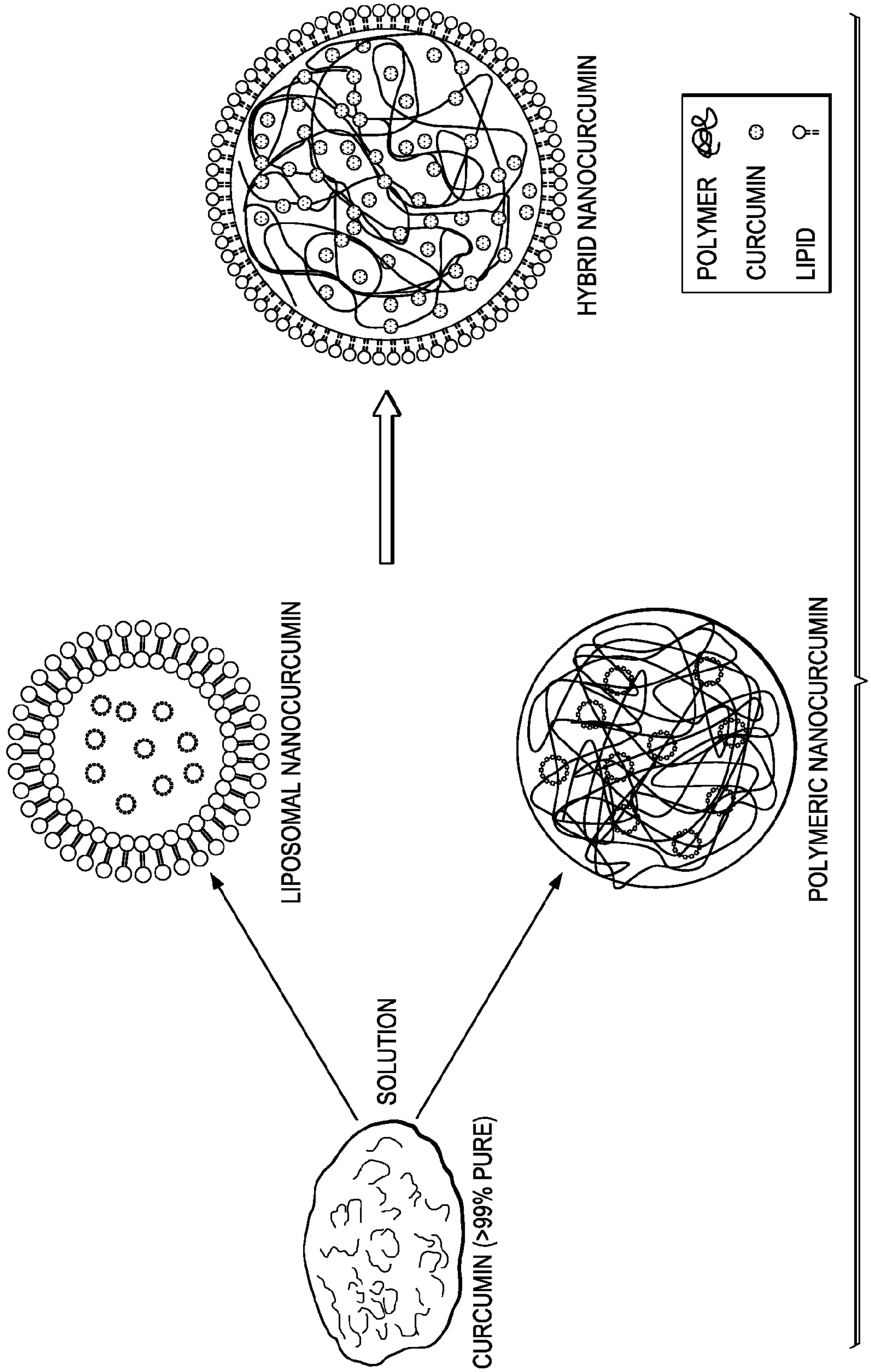

CURCUMIN (>99% PURE)
SOLUTION
LIPOSOMAL NANOCURCUMIN
POLYMERIC NANOCURCUMIN
HYBRID NANOCURCUMIN
POLYMER
CURCUMIN
LIPID

FIG. 1

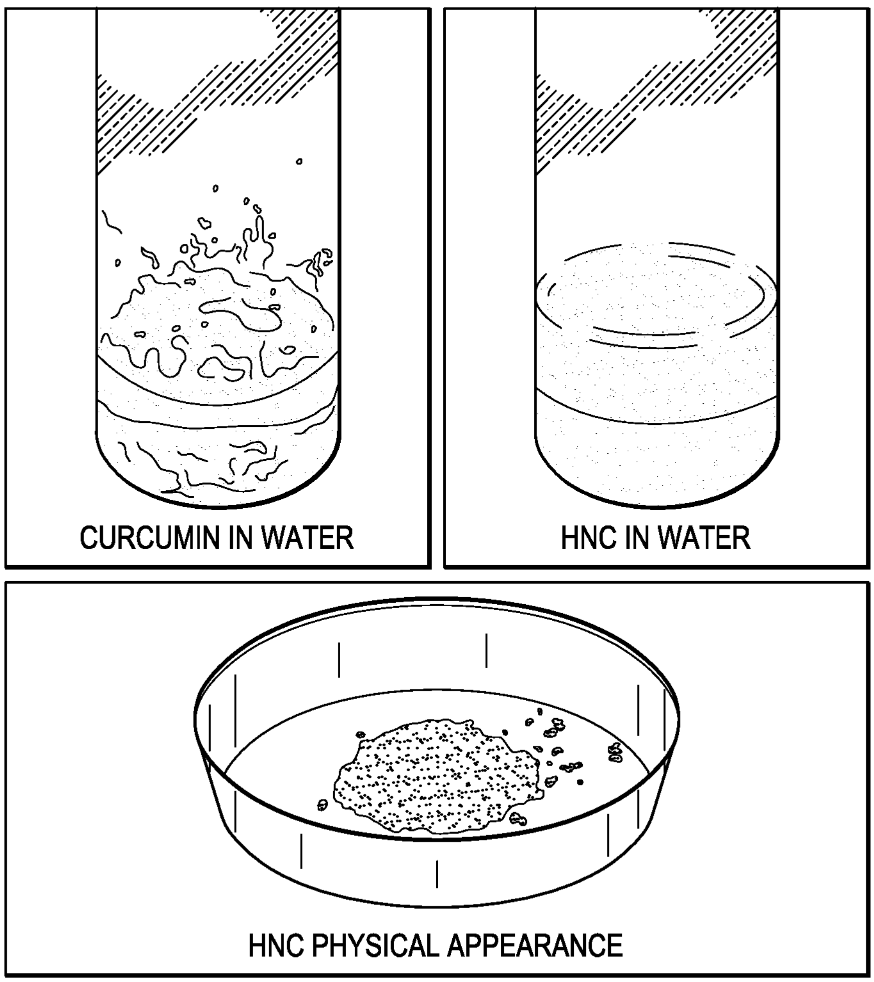


FIG. 2

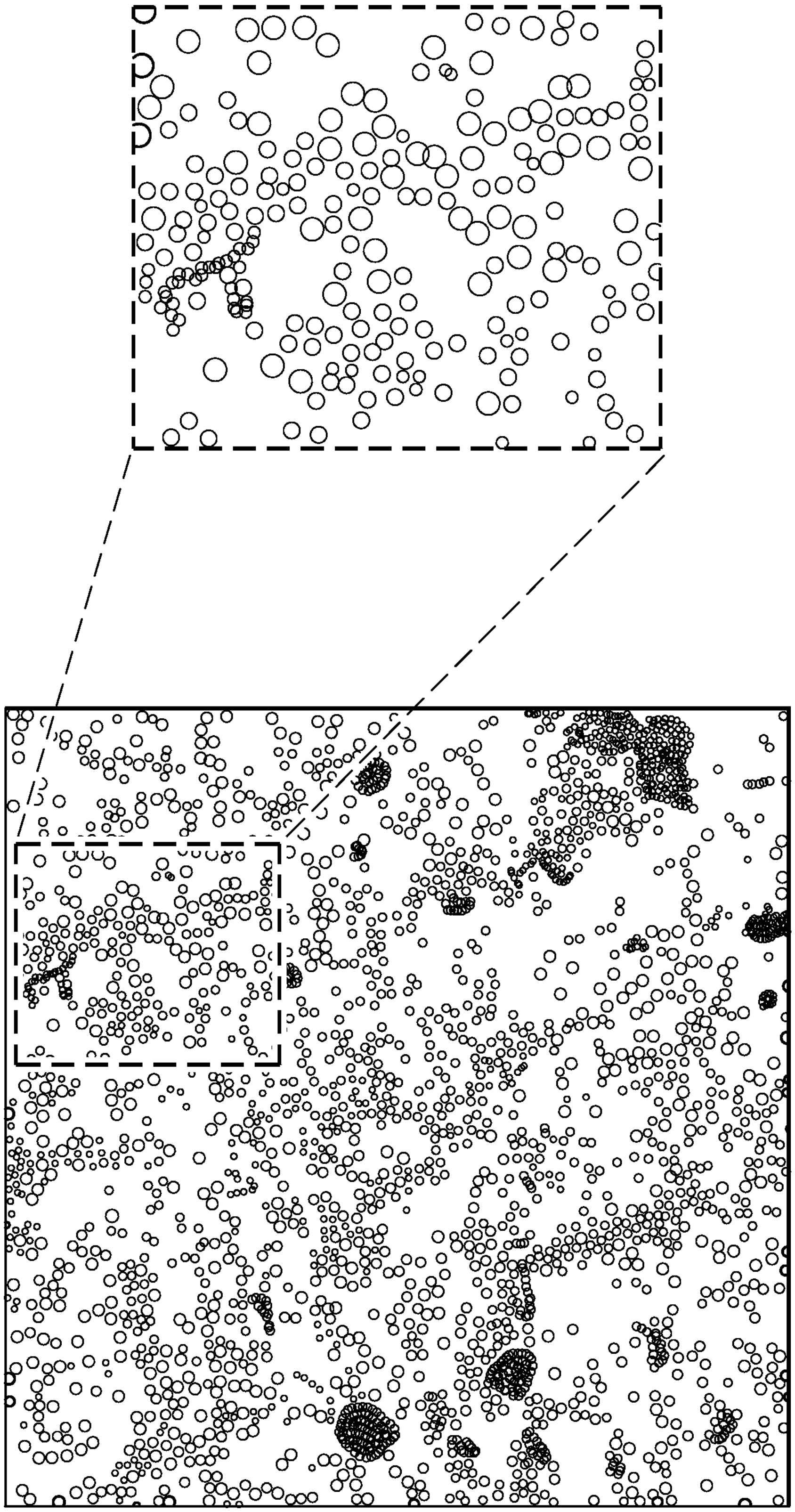


FIG. 3

EXP	FORMULATION FACTORS	1	2	3	4
A	PLGA CONCENTRATION (mg/ml)	10	10	10	10
B	$LIPID_1/LIPID_2$ MOLAR RATIO	7:3	7:3	7:3	7:3
C	ORGANIC/AQ VOLUME RATIO	1	1	1	1
D	DRUG LOADING (mg)	1	1	1	1
E	$(LIPID_1+LIPID_2)$-(mg)	2	4	6	8

FIG. 4A

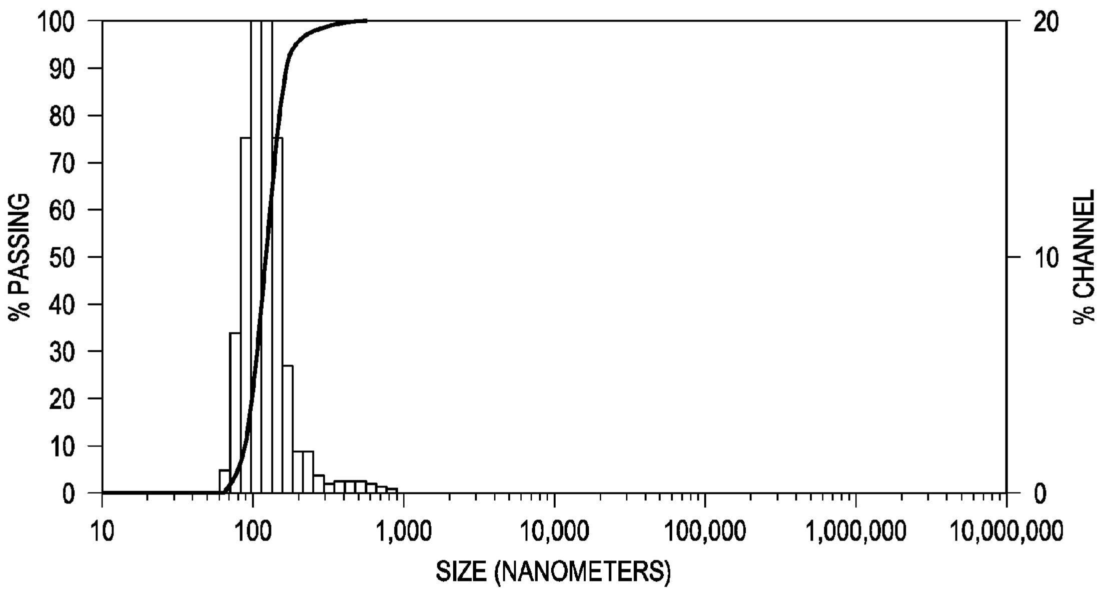


FIG. 4B

BATCH NUMBER	AVERAGE PARTICLE SIZE (nm)	DRUG LOADING (%)	ENCAPSULATION EFFICIENCY (%)
1	138.0	0.5	10
2	117.2	0.6	12
3	142.7	1.0	20
4	103.6	0.3	6

FIG. 5

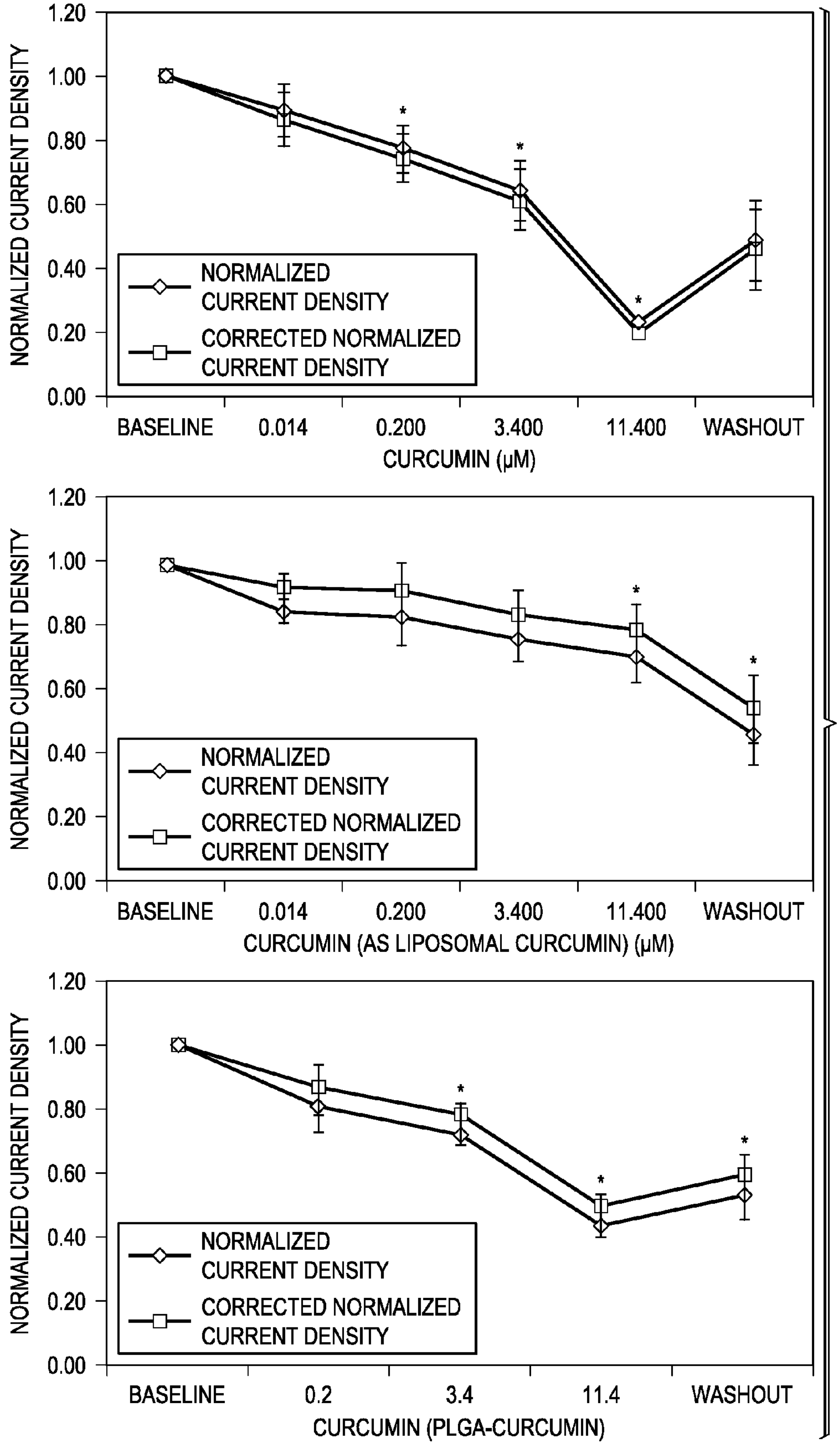
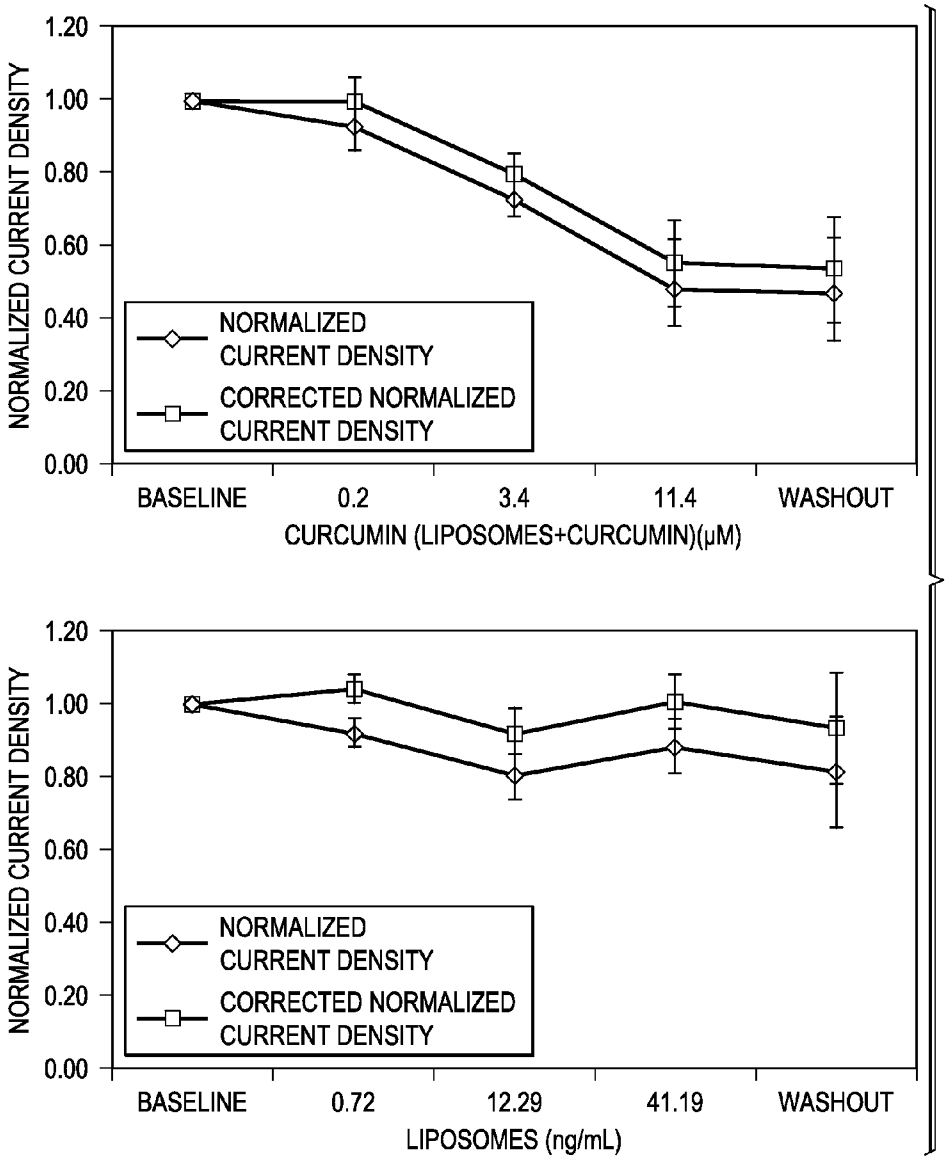

1.20
1.00
0.80
0.60
0.40
0.20
0.00
NORMALIZED CURRENT DENSITY
NORMALIZED CURRENT DENSITY
CORRECTED NORMALIZED CURRENT DENSITY
BASELINE
0.014
0.200
3.400
11.400
WASHOUT
CURCUMIN (μM)
CURCUMIN (AS LIPOSOMAL CURCUMIN) (μM)
0.2
3.4
11.4
CURCUMIN (PLGA-CURCUMIN)
*

FIG. 6



1.20
1.00
0.80
0.60
0.40
0.20
0.00
NORMALIZED CURRENT DENSITY
NORMALIZED CURRENT DENSITY
CORRECTED NORMALIZED CURRENT DENSITY
BASELINE
0.2
3.4
11.4
WASHOUT
CURCUMIN (LIPOSOMES+CURCUMIN)(μM)
1.20
1.00
0.80
0.60
0.40
0.20
0.00
NORMALIZED CURRENT DENSITY
NORMALIZED CURRENT DENSITY
CORRECTED NORMALIZED CURRENT DENSITY
BASELINE
0.72
12.29
41.19
WASHOUT
LIPOSOMES (ng/mL)

FIG. 7

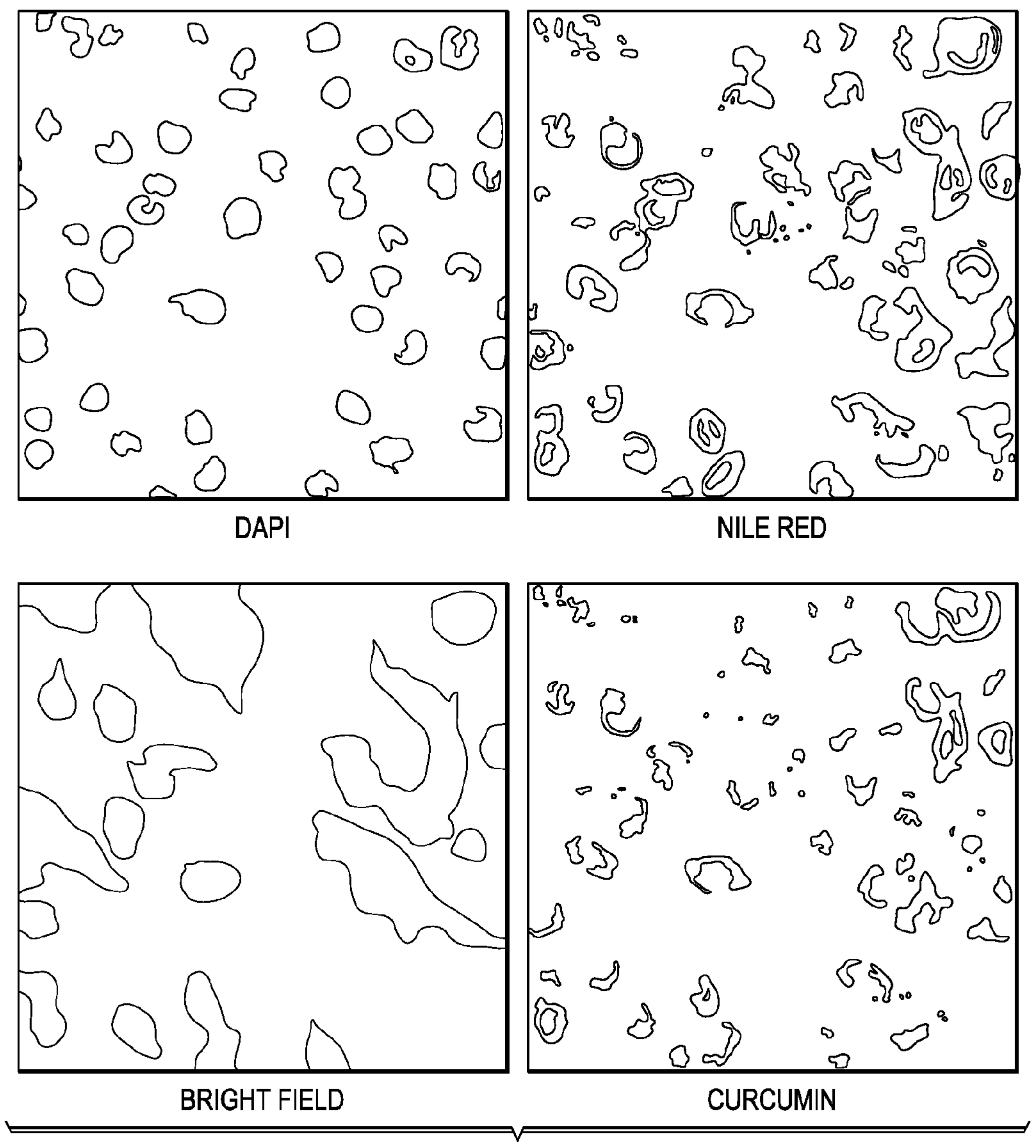


FIG. 8

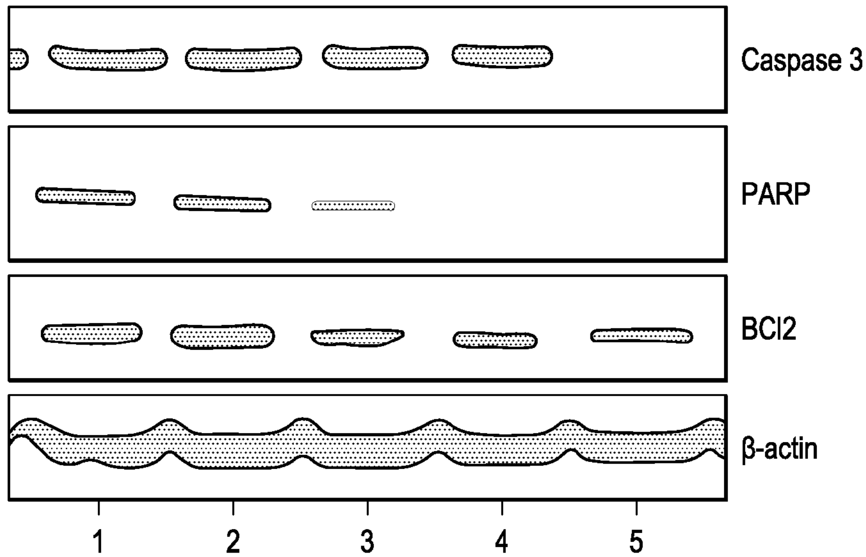

Caspase 3
PARP
BCl2
β-actin
1
2
3
4
5

FIG. 9

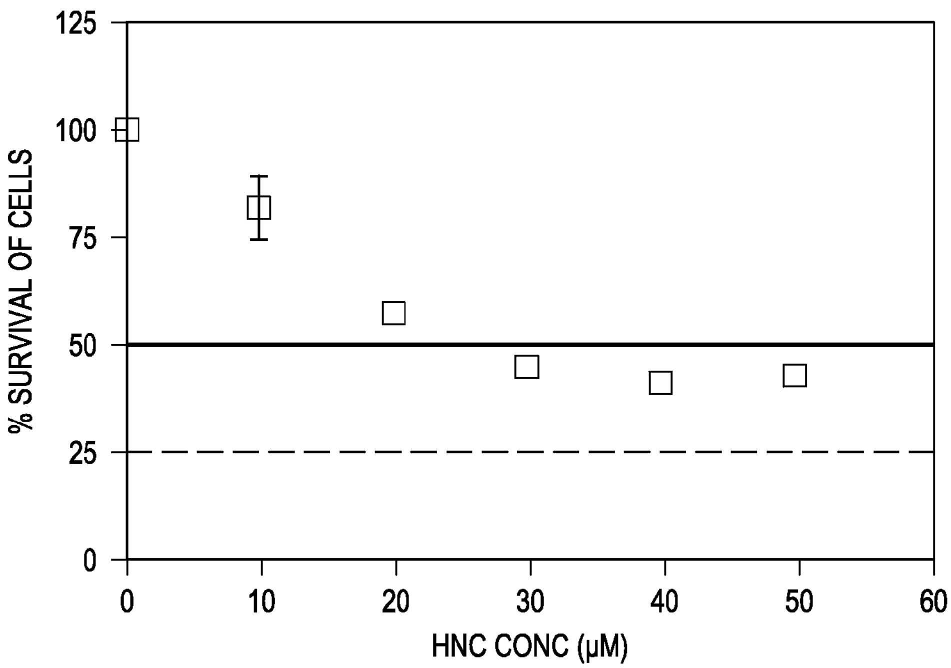

% SURVIVAL OF CELLS
125
100
75
50
25
0
0
10
20
30
40
50
60
HNC CONC (μM)

FIG. 10

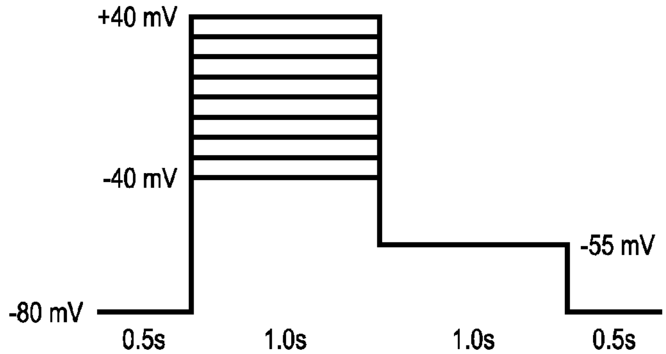


FIG. 11

	NORMALIZED CURRENT DENSITY	CORRECTED NORMALIZED CURRENT DENSITY	SEM	p value	n=
BASELINE	1.000	1.000	N/A	N/A	3
BATCH A, 6 μM	0.718	0.751	0.065	0.063	3
BATCH A, 12 μM	0.732	0.766	0.065	0.069	3
BATCH A, 18 μM	0.718	0.752*	0.030	0.014	3
WASHOUT	0.439	0.473	0.027	0.175	2

FIG. 12

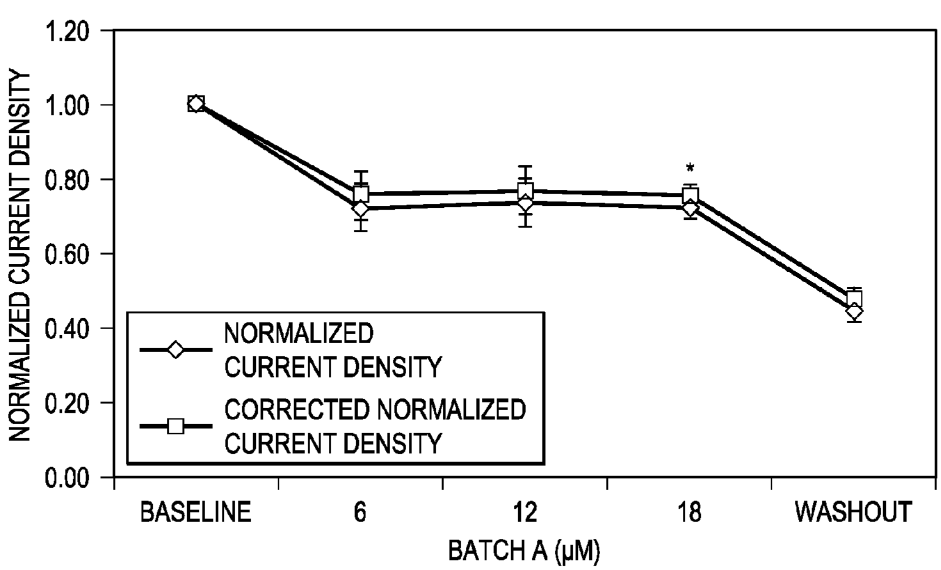


FIG. 13

FIG. 14

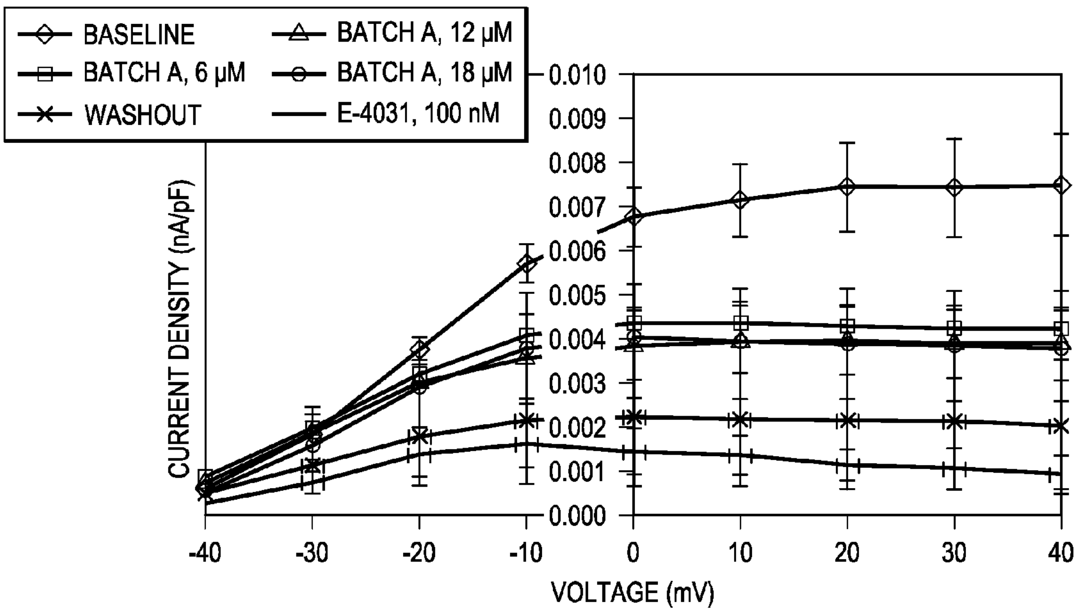


FIG. 15

	NORMALIZED CURRENT DENSITY	CORRECTED NORMALIZED CURRENT DENSITY	SEM	p value	n=
BASELINE	1.000	1.000	N/A	N/A	3
BATCH B, 6 μM	0.840	0.873*	0.019	0.023	3
BATCH B, 12 μM	0.877	0.911	0.133	0.572	3
BATCH B, 18 μM	0.565	0.598*	0.092	0.022	4
WASHOUT	0.460	0.493	0.086	0.473	2

FIG. 16

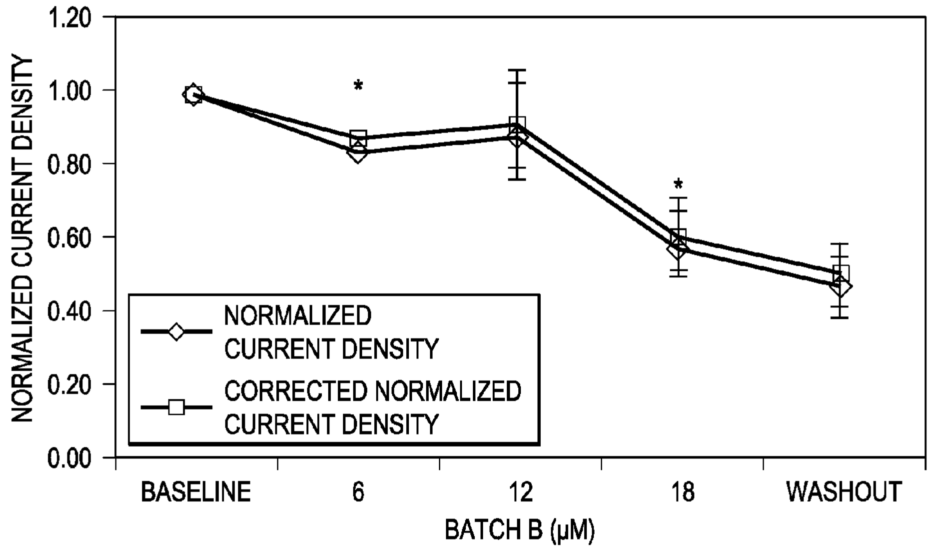

1.20
1.00
0.80
0.60
0.40
0.20
0.00
NORMALIZED CURRENT DENSITY
*
*
NORMALIZED CURRENT DENSITY
CORRECTED NORMALIZED CURRENT DENSITY
BASELINE
6
12
18
WASHOUT
BATCH B (μM)

FIG. 17

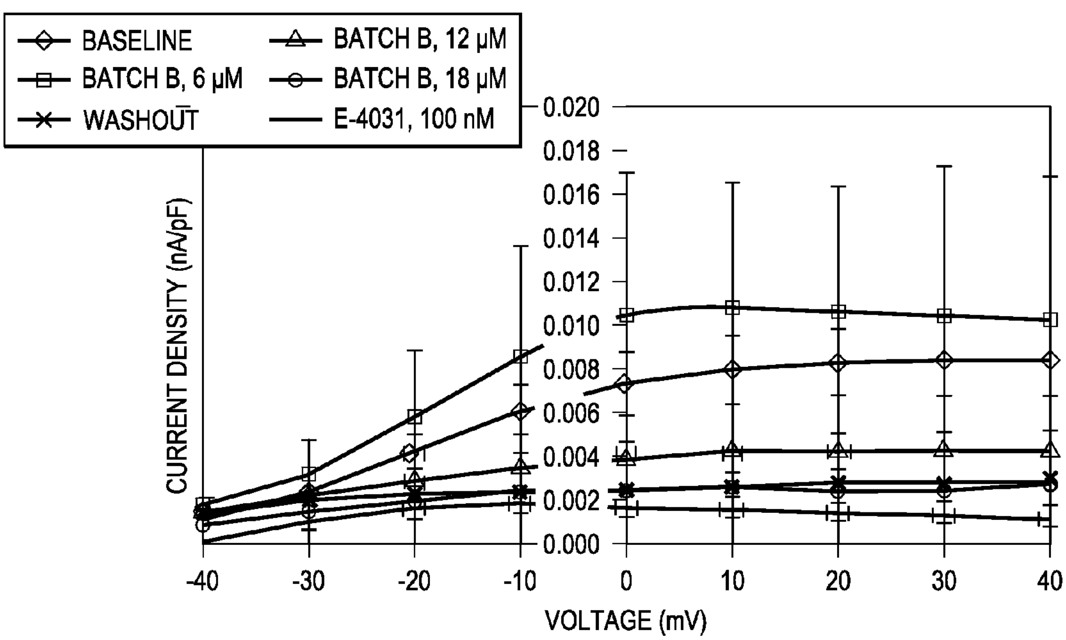

BASELINE
BATCH B, 6 μM
WASHOUT
BATCH B, 12 μM
BATCH B, 18 μM
E-4031, 100 nM
CURRENT DENSITY (nA/pF)
0.020
0.018
0.016
0.014
0.012
0.010
0.008
0.006
0.004
0.002
0.000
-40
-30
-20
-10
0
10
20
30
40
VOLTAGE (mV)

FIG. 18

	NORMALIZED CURRENT DENSITY	CORRECTED NORMALIZED CURRENT DENSITY	SEM	p value	n=
BASELINE	1.000	1.000	N/A	N/A	3
BATCH C, 6 μM	0.746	0.780*	0.039	0.029	3
BATCH C, 12 μM	0.754	0.787*	0.030	0.019	3
BATCH C, 18 μM	0.715	0.748*	0.045	0.031	3
WASHOUT	0.470	0.503	0.057	0.053	2

FIG. 19

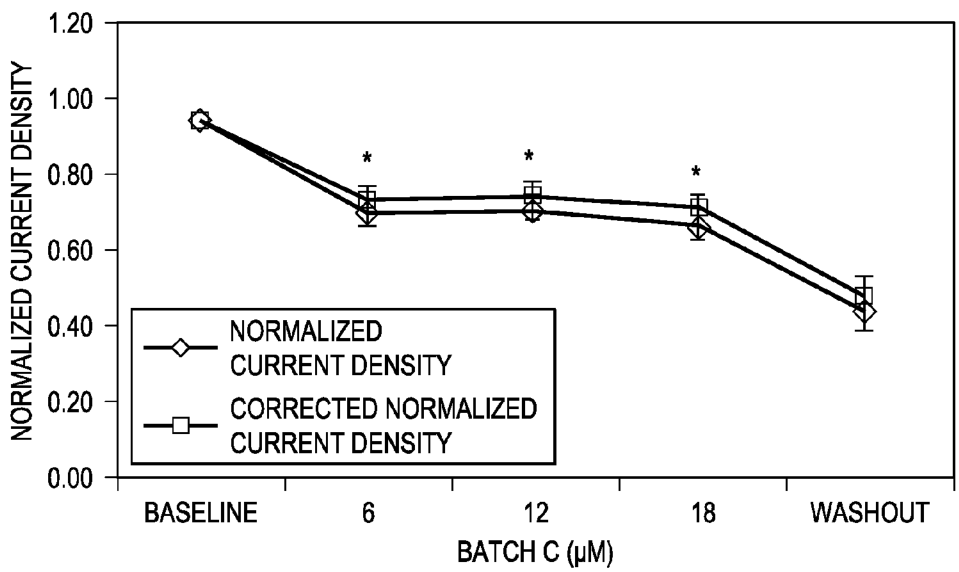


FIG. 20

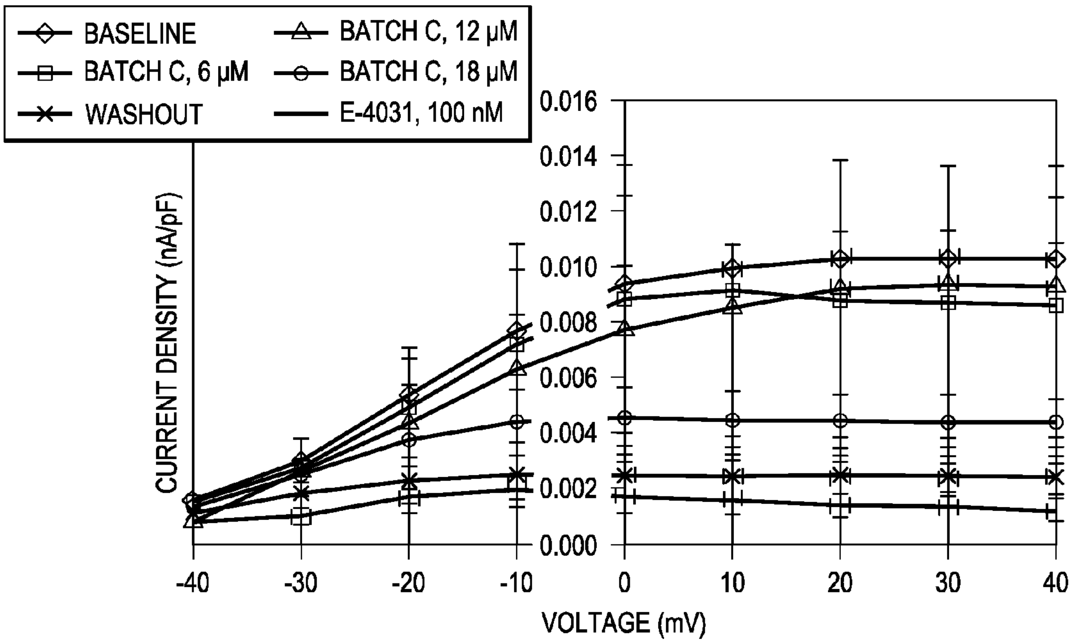


FIG. 21

	NORMALIZED CURRENT DENSITY	CORRECTED NORMALIZED CURRENT DENSITY	SEM	p value	n=
BASELINE	1.000	1.000	N/A	N/A	3
BATCH D, 6 μM	0.566	0.664	0.094	0.070	3
BATCH D, 12 μM	0.255	0.354*	0.046	0.005	3
BATCH D, 18 μM	0.124	0.222*	0.012	0.000	3
WASHOUT	0.277	0.376	0.131	0.485	2

FIG. 22

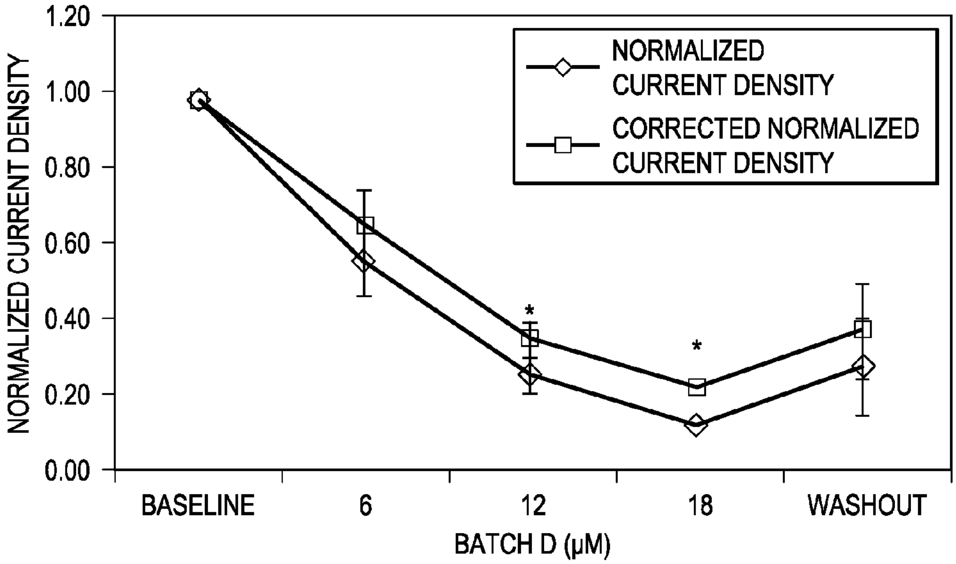

1.20
1.00
0.80
0.60
0.40
0.20
0.00
NORMALIZED CURRENT DENSITY
NORMALIZED CURRENT DENSITY
CORRECTED NORMALIZED CURRENT DENSITY
*
*
BASELINE
6
12
18
WASHOUT
BATCH D (μM)

FIG. 23

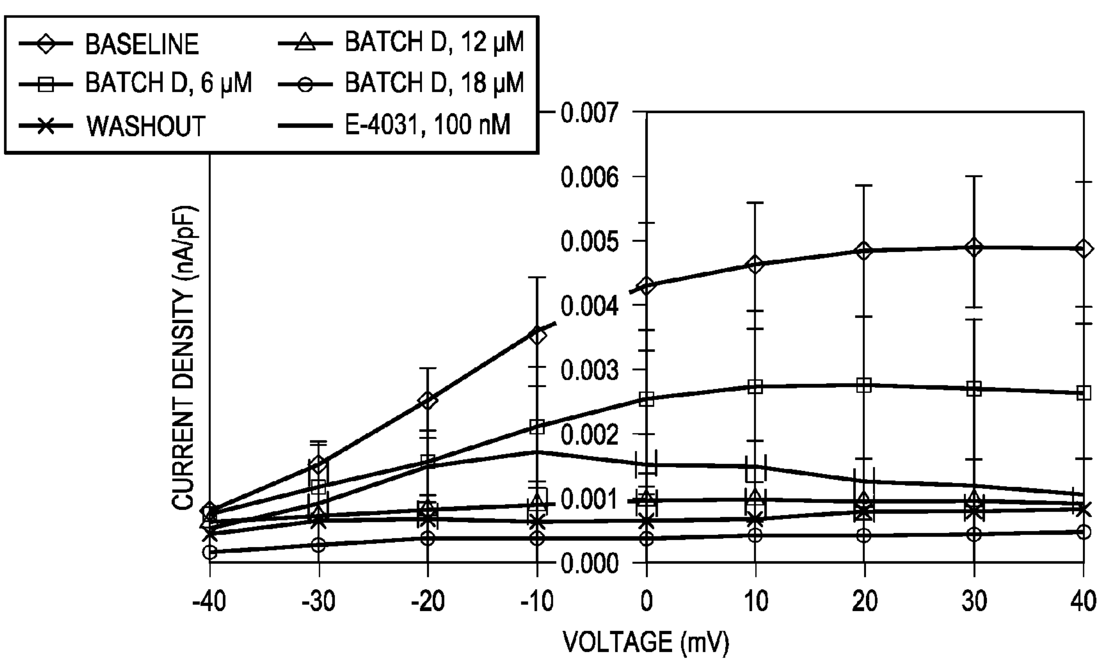

BASELINE
BATCH D, 6 μM
WASHOUT
BATCH D, 12 μM
BATCH D, 18 μM
E-4031, 100 nM
CURRENT DENSITY (nA/pF)
0.007
0.006
0.005
0.004
0.003
0.002
0.001
0.000
-40
-30
-20
-10
0
10
20
30
40
VOLTAGE (mV)

FIG. 24

	NORMALIZED CURRENT DENSITY	CORRECTED NORMALIZED CURRENT DENSITY	SEM	p value	n=
BASELINE	1.000	1.000	N/A	N/A	3
BATCH E, 6 μM	0.666	0.700*	0.041	0.018	3
BATCH E, 12 μM	0.810	0.844	0.049	0.087	3
BATCH E, 18 μM	0.813	0.847	0.070	0.161	3
WASHOUT	0.628	0.662	0.181	0.275	2

FIG. 25

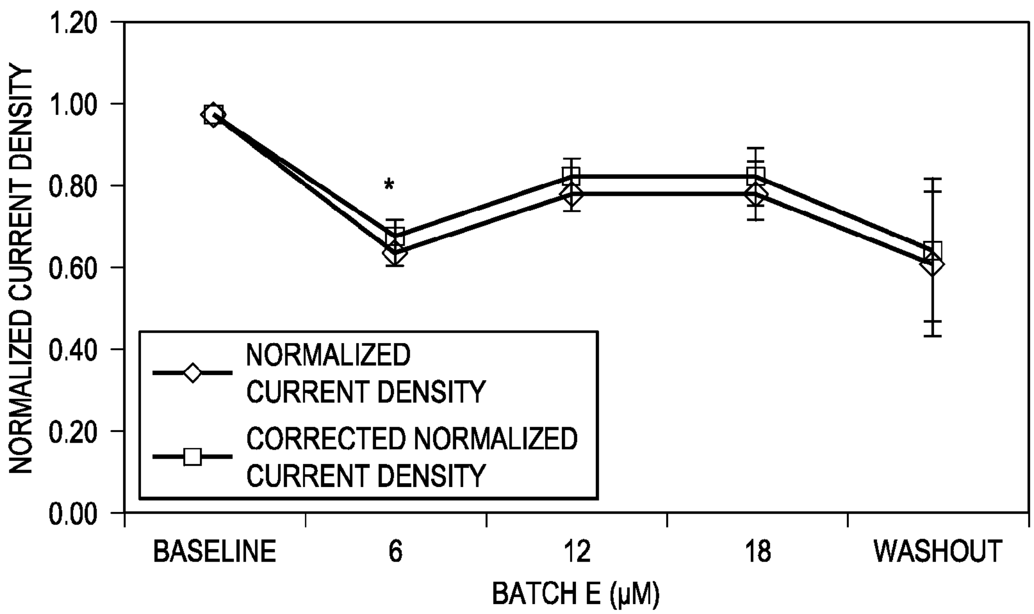


FIG. 26

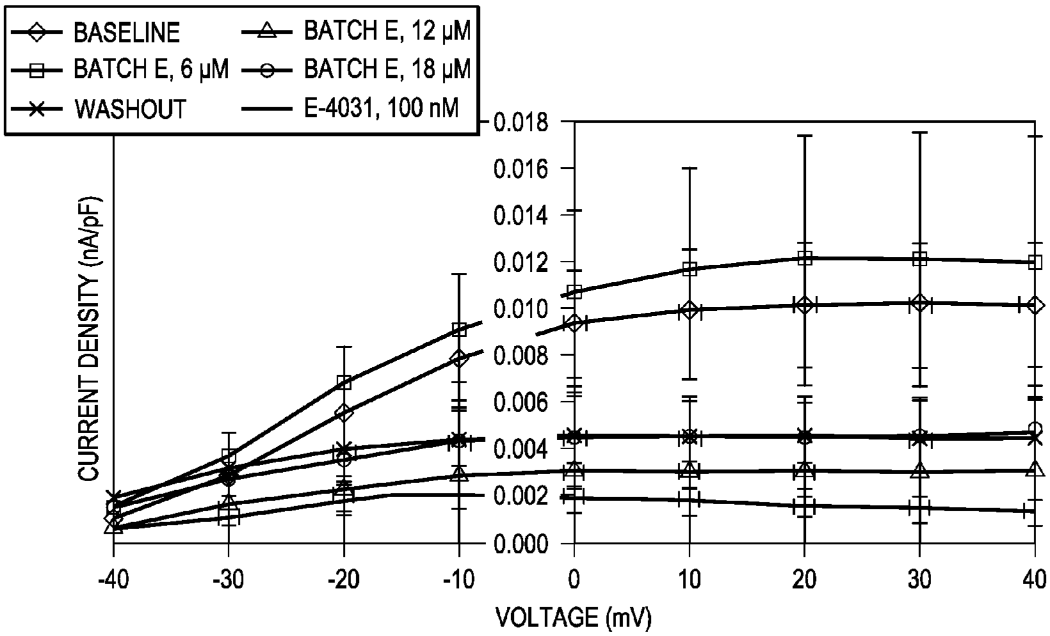


FIG. 27

COMPOUND TESTED	STATISTICALLY SIGNIFICANT INHIBITION STARTING AT (μM)	MAXIMAL INHIBITION (%)	STATISTICALLY SIGNIFICANT REVERSIBILITY OF THE WASHOUT	CALCULATED IC_{50} (μM)	VOLTAGE DEPENDENCE
BATCH A	18	24.8	NO	N/A	YES
BATCH B	6	40.2	NO	N/A	YES
BATCH C	6	25.2	NO	N/A	YES
BATCH D	12	77.8	NO	8.5	YES
BATCH E	6 ONLY	30.0	NO	N/A	NO

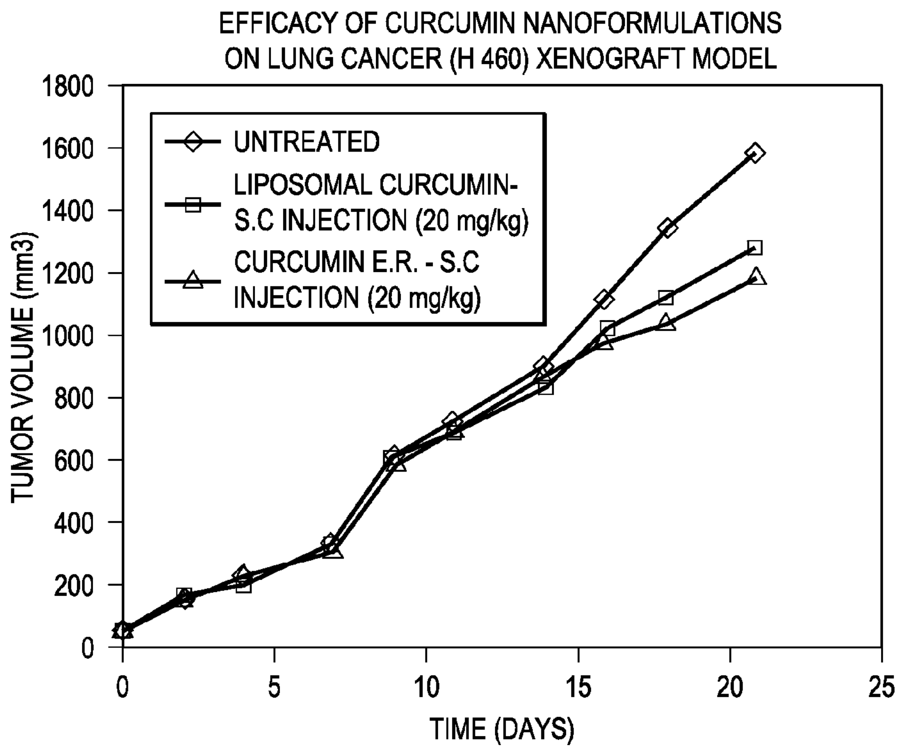

EFFICACY OF CURCUMIN NANOFORMULATIONS
ON LUNG CANCER (H 460) XENOGRAFT MODEL
TUMOR VOLUME (mm3)
1800
1600
1400
1200
1000
800
600
400
200
0
UNTREATED
LIPOSOMAL CURCUMIN-
S.C INJECTION (20 mg/kg)
CURCUMIN E.R. - S.C
INJECTION (20 mg/kg)
0
5
10
15
20
25
TIME (DAYS)

FIG. 28

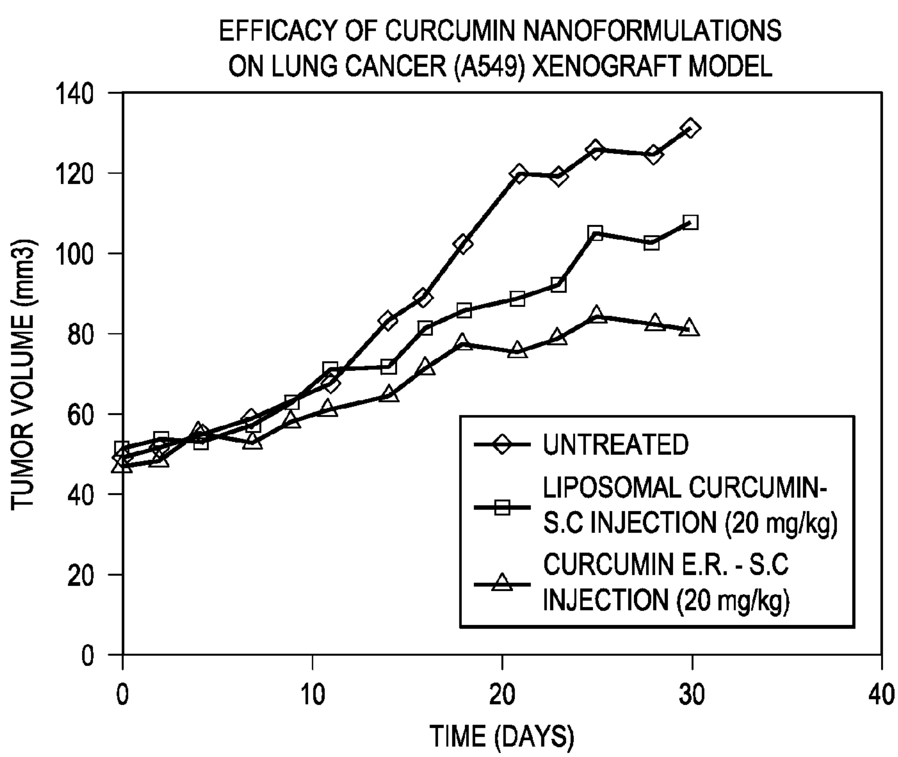

EFFICACY OF CURCUMIN NANOFORMULATIONS
ON LUNG CANCER (A549) XENOGRAFT MODEL
TUMOR VOLUME (mm3)
140
120
100
80
60
40
20
0
UNTREATED
LIPOSOMAL CURCUMIN-
S.C INJECTION (20 mg/kg)
CURCUMIN E.R. - S.C
INJECTION (20 mg/kg)
0
10
20
30
40
TIME (DAYS)

FIG. 29

CURCUMIN-ER, A LIPOSOMAL-PLGA SUSTAINED RELEASE NANOCURCUMIN FOR MINIMIZING QT PROLONGATION FOR CANCER THERAPY

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. Provisional Application Ser. No. 61/695,827, filed Aug. 31, 2012, the entire contents of which are incorporated herein by reference.

TECHNICAL FIELD OF THE INVENTION

The present invention relates in general to nanoparticles comprising a polymeric core comprising one or more polymers and one or more active agents and at least one layer of one or more lipids on the surface of the polymeric core. More specifically, the invention relates to the use of curcumin within such a lipid-polymer nanoparticle formulation for minimizing QT prolongation associated with curcumin in treatment of cancer.

STATEMENT OF FEDERALLY FUNDED RESEARCH

None.

BACKGROUND OF THE INVENTION

Without limiting the scope of the invention, its background is described in connection with the delivery of active pharmaceutical agents.

U.S. Pat. No. 7,968,115 to Kurzrock (filed Sep. 7, 2005) is said to provide a compositions and methods for the treatment of cancer, including pancreatic cancer, breast cancer and melanoma, in a human patient. The methods and compositions of the present invention employ curcumin or a curcumin analogue encapsulated in a colloidal drug delivery system, preferably a liposomal drug delivery system. Suitable colloidal drug delivery systems also include nanoparticles, nanocapsules, microparticles or block copolymer micelles. The colloidal drug delivery system encapsulating curcumin or a curcumin analogue is administered parenterally in a pharmaceutically acceptable carrier.

U.S. Pat. No. 8,202,839 to Sung (filed Jan. 7, 2012) is said to disclose a pharmaceutical composition of bioactive nanoparticles composed of chitosan, poly-glutamic acid, and a bioactive agent for oral delivery. The chitosan-based nanoparticles are characterized with a positive surface charge and enhanced permeability for oral drug delivery.

U.S. Patent Application Publication Number 20120058208 by Jacob (Synergistic Composition for Enhancing Bioavailability of Curcumin) (filed Mar. 8, 2012) is said to relate to a composition to enhance the bioavailability of curcumin. In one embodiment, a composition comprising plant extracts of curcumin, vanilla and ginger, wherein the extracts of ginger and vanilla are rich in gingerol and vanillin respectively, is provided. In other embodiments, curcumin, and one or more items selected from the group of vanilla, ginger and capsaicin is provided.

U.S. Patent Application Publication Number 20120003177 by Shen (Curcumin-containing polymers and water-soluble curcumin derivatives as prodrugs of prodrug carriers) (filed Jan. 5, 2012) is said to describe Curcumin, a polyphenol extracted from the rhizome turmeric, that has been polymerized to produce a polymer material having a backbone of one or more repeating structural units, at least one of which comprises a curcumin monomer residue. These curcumin-containing polymers have a wide range of pharmacological activities, including, among others antitumor, antioxidant, anti-inflammatory, antithrombotic and antibacterial activities. Certain species of these polymers have exhibited remarkable antitumor activity. Water-soluble curcumin derivatives and their use as prodrugs and prodrug carriers are also disclosed.

SUMMARY OF THE INVENTION

Problems associated with Curcumin are low solubility, low bioavailability, QT prolongation, and fast in vivo clearance. The advantages of liposomal nanocurcumin are no QT prolongation, high bioavailability, and low in vivo clearance, but the disadvantages are rapid release. The advantages of polymeric nanocurcumin are high bioavailability, sustained release, and low in vivo clearance, but the disadvantages are QT prolongation. The advantages of hybrid nanocurcumin are high bioavailability, sustained release, no QT prolongation, and low in vivo clearance.

The present invention includes methods and compositions comprising a polymeric nanoparticle core comprising one or more polymers and one or more active agents; and at least one layer of one or more lipids on the surface of the polymeric core. The one or more polymers may comprise PLGA; and/or at least one polymer selected from the group consisting of poly(lactic acid), polylactide (PLA), and poly-L-lactide-co-ϵ-caprolactone (PLCL). In certain aspects, the one or more active agents comprise curcumin or a curcuminoid. The active agent may comprise at least one anti-cancer drug; and/or be selected from at least one of an anti-cancer drug, an antibiotic, an antiviral, an antifungal, an antihelminthic, a nutrient, a small molecule, a siRNA, an antioxidant, and an antibody. In certain aspects, the nanoparticle composition does not cause QT prolongation. In certain aspects, the nanoparticle composition has high bioavailability. In certain aspects, the active agent may comprise a conventional radioisotope. The one or more active agents comprise a water-insoluble dye; and/or a metal nanoparticle, to be used as contrast agents for MRI; and/or be selected from the group comprising Nile red, iron, and platinum. In certain aspects, the one or more lipids comprise 1,2-Dimyristoyl-sn-glycero-3-phosphocholine (DMPC); and/or dimyristoyl phosphatidylglycerol (DMPG); 1,2-dioctadecanoyl-sn-glycero-3-phosphoethanolamine (DSPE), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[amino(polyethylene glycol) (DSPE-PEG), DMPE PEG Maleimide, Lecithin, cholesterol, 1,2-dimyristoyl-sn-glycero-3-phosphoethanolamine-N-(lissamine rhodamine B sulfonyl) (ammonium salt), and 1,2-dimyristoyl-sn-glycero-3-phosphoethanolamine-N-(7-nitro-2-1,3-benzoxadiazol-4-yl) (ammonium salt). In various aspects, the nanoparticle composition may comprise DMPC and DMPG in a molar ratio of 9:1, 7:3, 8:2, or 7.5:2.5. In certain aspects, the nanoparticles may comprise at least one targeting agent, wherein the targeting agent selectively targets the nanoparticle to diseased tissue/cells, thereby minimizing whole body dose; and/or wherein the targeting agent comprises an antibody or functional fragment thereof that is capable of recognizing a target antigen; and/or selected from the group consisting of an antibody, a small molecule, a peptide, a carbohydrate, an siRNA, a protein, a nucleic acid, an aptamer, a second nanoparticle, a cytokine, a chemokine, a lymphokine, a receptor, a lipid, a lectin, a ferrous metal, a magnetic particle, a linker, an isotope and combinations thereof. In certain aspects, the nanoparticles have a size of 90 to 150 nm. The bioavailability of the active agent may be increased, a QT prolongation is reduced, and the active agent may be released in a sustained manner.

The invention includes embodiments of methods for forming a nanoparticle composition comprising forming an organic phase by combining one or more polymers, one or more solvents and one or more active agents; forming a lipid aqueous phase by mixing one or more lipids with water; mixing the organic phase with the lipid aqueous phase, whereby an emulsion is formed; and incubating the emulsion, whereby self-assembly of nanoparticles occurs. The one or more polymers may comprise PLGA; and/or at least one polymer selected from the group consisting of poly(lactic acid), polylactide (PLA), and poly-L-lactide-co-ϵ-caprolactone (PLCL). The organic phase may comprise PLGA in a concentration of 2-90 mg/ml; and/or curcumin in a concentration of 1-15 weight/volume %. In various aspects, the one or more solvents may comprise an organic solvent; acetonitrile; at least one solvent selected from the group consisting of Acetone, tert butyl alcohol, Dimethyl formamide, and Hexafluoro isopropanol. The one or more active agents comprise curcumin or a curcuminoid; and/or at least one anticancer drug; and/or a conventional radioisotope; and/or at least one active agent selected from the group consisting of selected from the group comprising Nile red, iron, and platinum. In certain aspects, the one or more lipids may comprise DMPC; and/or DMPG, and/or at least one lipid selected from the group consisting of 1,2-dioctadecanoyl-sn-glycero-3-phosphoethanolamine (DSPE), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[amino(polyethylene glycol) (DSPE-PEG), DMPE PEG Maleimide, Lecithin, cholesterol, 1,2-dimyristoyl-sn-glycero-3-phosphoethanolamine-N-(lissamine rhodamine B sulfonyl) (ammonium salt), and 1,2-dimyristoyl-sn-glycero-3-phosphoethanolamine-N-(7-nitro-2-1,3-benzoxadiazol-4-yl) (ammonium salt). In certain aspects, the one or more lipids comprise DMPC and DMPG in a molar ratio of 9:1, 7:3, 8:2, 7.5:2.5. In certain aspects, mixing the organic phase with the lipid aqueous phase comprises slowly stirring the organic phase into the lipid aqueous phase; and/or mixing the organic phase with the lipid aqueous phase comprises vortexing; and/or mixing the organic phase with the lipid aqueous phase further comprises sonicating. In certain aspects, incubating the emulsion comprises stirring the emulsion for 2 hours. In certain aspects, the method may further comprise separating the nanoparticles after incubating the emulsion; and/or filtering the nanoparticles after incubating the emulsion; and/or freezing the nanoparticles; and/or lyophilizing the nanoparticles; and/or attaching a targeting agent to the nanoparticles; and/or attaching at least one targeting agent, wherein the targeting agent selectively targets the nanoparticle to diseased tissue/cells, thereby minimizing whole body dose; and/or attaching at least one targeting agent to the nanoparticles, wherein the targeting agent comprises an antibody or functional fragment thereof that is capable of recognizing a target antigen. In certain aspects, the nanoparticles have a size of 90 to 150 nm.

The invention includes embodiments of pharmaceutical agents comprising a nanoparticle for drug delivery comprising a polymer, an active agent and at least one layer of one or more lipids encapsulating the polymer and the active agent.

The invention includes embodiments for treating a patient suspected of being afflicted with a disease comprising administering nanoparticles, wherein the nanoparticles comprise a polymeric core comprising one or more polymers and one or more active agents and at least one layer of one or more lipids on the surface of the polymeric core. In certain aspects, administering nanoparticles comprises administering the nanoparticle by intramuscular, subcutaneous, intravascular, or intravenous administration. Disease can be selected from the group consisting of neurologic, oncologic, and metabolic diseases; and/or from the group consisting of Parkinson's disease, Alzheimer's disease, multiple sclerosis, ALS, sequel, behavioral and cognitive disorders, autism spectrum, depression, and neoplastic disease; and/or cancer. In certain aspects, the active agent is released in a sustained manner.

The invention includes embodiments of composition comprising a polymeric nanoparticle core comprising one or more polymers and curcumin and at least one layer of one or more lipids on the surface of the polymeric core.

The invention includes embodiments of forming a nanoparticle composition comprising forming an organic phase by combining one or more polymers, one or more solvents and curcumin; forming a lipid aqueous phase by mixing one or more lipids with water; mixing the organic phase with the lipid aqueous phase, whereby an emulsion is formed; and incubating the emulsion, whereby self-assembly of nanoparticles occurs.

The invention includes embodiments of pharmaceutical agents comprising a nanoparticle for drug delivery comprising a polymer, curcumin, and at least one layer of one or more lipids encapsulating the polymer and the active agent.

The invention includes embodiments of methods for treating a patient suspected of being afflicted with a disease, the method comprising administering nanoparticles, wherein the nanoparticles comprise a polymeric core comprising one or more polymers, curcumin, and at least one layer of one or more lipids on the surface of the polymeric core.

Another embodiment includes a composition for treating cancer comprising: a polymeric nanoparticle core comprising one or more polymers and at least one of curcumin or curcuminoids; and at least one layer of one or more lipids on the surface of the polymeric core, wherein the at least one of the curcumin or curcuminoids nanoparticles, wherein the composition does not cause QT prolongation when provided to a subject. In one aspect, the one or more polymers comprise at least one of poly(lactic-co-glycolic acid) (PLGA), poly(lactic acid), polylactide (PLA), or poly-L-lactide-co-ϵ-caprolactone (PLCL).

Another embodiment includes a method of forming a nanoparticle composition comprising: forming an organic phase by combining one or more polymers, one or more solvents and at least one of curcumin or curcuminoids; forming a lipid aqueous phase by mixing one or more lipids with water; mixing the organic phase with the lipid aqueous phase, whereby an emulsion is formed; and incubating the emulsion, whereby self-assembly of nanoparticles occurs and wherein the curcumin or curcuminoids nanoparticles does not cause QT prolongation when provided to a subject.

Another embodiment includes a method for treating a patient suspected of being afflicted with a disease comprising administering nanoparticles, wherein the nanoparticles comprise a polymeric core comprising one or more polymers and one or more active agents and at least one layer of one or more lipids on the surface of the polymeric core, wherein the active agent is suspected of causing QT prolongation when provided to a subject. In one aspect, the method also includes the step of administering the nanoparticle by intramuscular, subcutaneous, intravascular, or intravenous administration.

Another embodiment includes a method of forming a nanoparticle that prevents the active agent from causing QT prolongation composition comprising: forming an organic phase by combining one or more polymers, one or more solvents and the active agent that causes QT prolongation; forming a lipid aqueous phase by mixing one or more lipids with water; mixing the organic phase with the lipid aqueous phase, whereby an emulsion is formed; and incubating the emulsion, whereby self-assembly of nanoparticles occurs.

Another embodiment includes a pharmaceutical agent comprising: a nanoparticle for drug delivery comprising a polymer, an active agent that causes QT prolongation, and at least one layer of one or more lipids encapsulating the polymer and the active agent and the agent does not cause QT prolongation.

Another embodiment includes a method for treating a patient suspected of being afflicted with a disease, the method comprising administering nanoparticles, wherein the nanoparticles comprise a polymeric core comprising one or more polymers, curcumin, and at least one layer of one or more lipids on the surface of the polymeric core, wherein treating the patient does not cause QT prolongation.

In another embodiment, the method of treating a subject suspected of having cancer includes: identifying that a patient suspected of having a cancer; and Providing the subject with an amount of at least one or curcumin or curcuminoids in an amount sufficient to reduce the cancer in the subject, wherein the at least one or curcumin or curcuminoids are in a polymeric nanoparticle core comprising one or more polymers and at least one of curcumin or curcuminoids; and at least one layer of one or more lipids on the surface of the polymeric core, wherein the at least one of the curcumin or curcuminoids nanoparticles does not cause QT prolongation when provided to a subject. In one aspect, the cancer is a pancreatic, prostate or breast cancer.

BRIEF DESCRIPTION OF THE DRAWINGS

For a more complete understanding of the features and advantages of the present invention, reference is now made to the detailed description of the invention along with the accompanying figures and in which:

FIG. **1** depicts the basic concept of hybrid nanocurcumin (HNC) formation; Lipids-DMPC and DMPG. Problems associated with Curcumin are low solubility, low bioavailability, QT prolongation, and fast in vivo clearance. The advantages of liposomal nanocurcumin are no QT prolongation, high bioavailability, and low in vivo clearance, but the disadvantages are rapid release. The advantages of polymeric nanocurcumin are high bioavailability, sustained release, and low in vivo clearance, but the disadvantages are QT prolongation. The advantages of hybrid nanocurcumin are high bioavailability, sustained release, no QT prolongation, and low in vivo clearance.

FIG. **2** demonstrates improved dispersibility in water with HNC.

FIG. **3** represents transmission electron micrographs showing HNC. The TEM scan shows HNC as spherical smooth nanoparticles with uniform size.

FIGS. **4**A and **4**B: FIG. **4**A shows formulations of hybrid nanocurcumin (HNC). Demonstrated are four different formulations of HNC using different ratio of DMPC and DMPG. FIG. **4**B shows particle size distribution of Batch 3.

FIG. **5** shows HNC characterization, including average particle size, drug loading, and encapsulation efficiency.

FIG. **6** shows hERG current density analysis of curcumin; liposomal curcumin; and PLGA curcumin.

FIG. **7** shows hERG current density analysis of liposomes+curcumin; and liposomes.

FIG. **8** shows intracellular uptake of HNC in MiaPaCa cells.

FIG. **9** shows Western blot analysis of MiaPaCa cells treated with hybrid nanocurcumin (25 μM (micromolar)). Lane 1: Untreated; lane 2: Blank nanoparticle; lane 3: Curcumin (24 hrs); lane 4: HNC (24 hrs) and; lane 5 HNC (48 hrs).

FIG. **10** shows MTT cell viability using HNC employing a pancreatic cancer cell line (MiaPaCa cell line) at 48 hours.

FIG. **11** shows the pulses protocol or the original data acquisition design: Acquisition Rate(s): 1.0 kHz

FIG. **12** shows the effect of batch A on hERG current density from transfected HEK 293 cells at 20 my.

FIG. **13** shows the effect of batch A on hERG current density from transfected HEK 293 cells at 20 mV.

FIG. **14** shows the relationship (I-V) of hERG current amplitude from transfected HEK 293 cells exposed to Batch A.

FIG. **15** shows the effect of batch B on hERG current density from transfected HEK 293 cells at 20 mV.

FIG. **16** shows the effect of batch B on hERG current density from transfected HEK 293 cells at 20 mV.

FIG. **17** shows the relationship (I-V) of hERG current amplitude from transfected HEK 293 cells exposed to Batch B.

FIG. **18** shows the effect of batch C on hERG current density from transfected HEK 293 cells at 20 mV.

FIG. **19** shows the effect of Batch C on hERG current density from transfected HEK 293 cells at 20 mV.

FIG. **20** shows the relationship (I-V) of hERG current amplitude from transfected HEK 293 cells exposed to Batch C.

FIG. **21** shows the effect of batch D on hERG current density from transfected HEK 293 cells at 20 mV.

FIG. **22** shows the effect of batch D on hERG current density from transfected HEK 293 cells at 20 mV.

FIG. **23** shows the relationship (I-V) of hERG current amplitude from transfected HEK 293 cells exposed to Batch D.

FIG. **24** shows effect of batch E on hERG current density from transfected HEK 293 cells at 20 mV.

FIG. **25** shows the effect of batch E on hERG current density from transfected HEK 293 cells at 20 mV.

FIG. **26** shows relationship (I-V) of hERG current amplitude from transfected HEK 293 cells exposed to Batch E.

FIG. **27** shows the effect of tested compounds on hERG current density at +20 mV.

FIG. **28** shows the results of the treatment of breast cancer in a cancer xenograft mouse model system.

FIG. **29** shows additional results of the treatment of a different breast cancer in a cancer xenograft mouse model system.

DETAILED DESCRIPTION OF THE INVENTION

While the making and using of various embodiments of the present invention are discussed in detail below, it should be appreciated that the present invention provides many applicable inventive concepts that can be embodied in a wide variety of specific contexts. The specific embodiments discussed herein are merely illustrative of specific ways to make and use the invention and do not delimit the scope of the invention.

To facilitate the understanding of this invention, a number of terms are defined below. Terms defined herein have meanings as commonly understood by a person of ordinary skill in the areas relevant to the present invention. Terms such as "a", "an" and "the" are not intended to refer to only a singular entity, but include the general class of which a specific example may be used for illustration. The terminology herein is used to describe specific embodiments of the invention, but their usage does not delimit the invention, except as outlined in the claims.

Problems associated with Curcumin are low solubility, low bioavailability, QT prolongation, and fast in vivo clearance. The advantages of liposomal nanocurcumin are no QT prolongation, high bioavailability, and low in vivo clearance, but the disadvantages are rapid release. The advantages of polymeric nanocurcumin are high bioavailability, sustained release, and low in vivo clearance, but the disadvantages are QT prolongation. The advantages of hybrid nanocurcumin are high bioavailability, sustained release, no QT prolongation, and low in vivo clearance.

A requirement in commercial drug development is to assay drug effects on hERG (Ikr) in in vitro assays using transfected KEK293 cells. The present inventors determined anti-hERG activity of curcumin (diferuloylmethane) in DMSO, and of three formulated curcumin compounds: liposomal curcumin, nanocurcumin, and a sustained release PLGA curcumin. The present inventors recognize that the K+ current IC_{50} of curcumin formulated in DMSO is 3.4 uM. Considered within the context of current clinical Phase 1a pharmacokinetics in normal subjects where blood plasma levels range between 5-11 uMol, following a two hour infusion of 4.5 mg/kg, intravenous, or subcutaneous curcumin formulations for therapeutic applications can inhibit IKr, lead to Torsade de Points, and possible clinical mortality. However, neither the liposomal, nor the nanocurcumin formulation at 12 uMol exhibits this effect on the K+ channel. The co-administration of empty liposomes to curcumin was equally effective in prohibiting the hERG blockade, however, the PLGA-curcumin formulation lacked this effect.

These observations are one basis for (constructing) a new curcumin formulation consisting of liposome and PLGA, which allows sustained release of curcumin without the associated cardiac K+ channel inhibitory properties of curcumin.

The treatment of cancer is limited by the side effects of the anti-cancer drugs. Chemotherapy is the only available option for the treatment of advanced cancers. However, increasing evidences of drug resistance and non-specific toxicity of these agents limits their therapeutic outcomes. To overcome this problem it is important to deliver the drug at the site of cancer in the body in the right amount. A novel way to approach this problem is through targeted drug delivery system, which preferentially delivers the drug to the site of cancer. In certain embodiment, targeting molecules (e.g., antibodies) that recognize the cancer cells and direct the drug containing tiny spherical particles (nanoparticles) to the cancer cells are used.

In certain embodiments, at least one targeting agent is attached to the nanoparticles, wherein the targeting agent comprises an antibody or functional fragment thereof that is capable of recognizing a target antigen. The targeting agents may be attached by insertion of hetero/homo bifunctional spacer capable of reacting with amines of lipids and targeting moieties.

Curcumin is a potent anticancer agent and is being used for its pharmacological action for last few decades. However, the major problems associated with curcumin are (1) low systemic bioavailability following administration via any route; (2) curcumin alone brings about QT prolongation; and (3) fast in vivo clearance of curcumin. The present inventors solved these problems by formulating curcumin (99% pure) into a hybrid nanoformulation. See FIG. **1**.

The present inventors recognized that a nanoformulation provides the solutions to increase bioavailability and that liposome formulation of curcumin show almost no QT prolongation. But such formulations lack stability and possess some inherent toxicity at higher doses. The present inventors recognize that curcumin has a very rapid clearance when administered in animal models.

The present inventors have developed a nanoformulation system that increases the bioavailability of curcumin, minimizes the QT prolongation, and releases the drug curcumin in a sustained manner.

The hybrid nanocurcumin (HNC) system is a hybrid of lipids and polymer wherein the polymeric core encapsulates curcumin. The lipid is present as a continuous layer on the surface of the polymeric nanoparticle. In other word, the lipid cases the polymeric nanoparticle. The lipid component of the hybrid nanocurcumin helps in reducing the QT prolongation while the polymeric core of the hybrid system facilitates the release of curcumin in a sustained manner. The hybrid nanocurcumin (HNC) system solved all the above-mentioned problems of (1) bioavailability of curcumin, (2) QT prolongation due to curcumin and (3) sustained release of curcumin simultaneously.

The advantages of hybrid nanocurcumin (HNC) system are: (1) in vivo bioavailability of active agents (e.g., curcumin) is improved; (2) the lipid component of the hybrid nanocurcumin reduces QT prolongation; (3) the polymeric core of the hybrid system facilitates the release of the active agent (e.g., curcumin) in a sustained manner; (4) the formulation itself is simple, convenient one-step process; and (5) this system can be used to formulate other similar type of drugs or active agents, which may comprise hydrophobic molecules. Examples would include curcumin analogues, docetaxel, paclitaxel etc.

The commercial potentials of hybrid nanocurcumin formulation are enormous due to better bioavailability and reduced side effects.

An embodiment is a Liposomal-Curcumin-PLGA sustained release compound for prevention and treatment of neurologic, oncologic, or metabolic diseases (Hybrid Nanocurcumin formulation).

Certain embodiments can be described as intravenous and/or subcutaneous administration of a novel formulation of synthesized curcumin (diferuloylmethane) bound to PLGA and a liposome. Such formulation is designed to offer a sustained release of curcumin as active agent. Reference is made to the prevention of cardiac events due to the incorporation of a liposomal component of the formulation.

In further embodiments the compositions may be used for the treatment of neurologic-auto-immunological degenerative diseases (Parkinson's disease, Alzheimer's disease, multiple sclerosis, ALS, sequel, behavioral and cognitive disorders, autism spectrum, and depression), neoplastic diseases (cancer).

In certain embodiments the compositions of the present invention are administered intramuscular, subcutaneous and or intravascular.

Certain embodiments comprise curcumin (diferuloylmethane)-encapsulated in a liposomal-PLGA envelope designated hybrid nanocurcumin formulation.

In one embodiment, the active agent is curcumin, which is a potent natural anticancer agent, is employed in a nanoparticle-based delivery system. One limitation is the QT prolongation effect of curcumin, even when it is associated with nanoparticle-based systems. This makes it difficult to pass FDA standards for commercial use. The hybrid nanocurcumin formulation solves this problem and reduces QT prolongation effect of curcumin, which makes it ideal for commercial application. In addition, the hybrid nanocurcumin formulation releases curcumin in a sustained manner, which improves the systemic availability and decreases fast clearance of curcumin in animal models. Therefore, the hybrid nanocurcumin formulation can directly be used to produce nanotechnology based hybrid dosage forms for curcumin. In other embodiments curcumin may be replaced by a variety of similar drugs or active agents. Such compositions may directly go into production by pharmaceutical companies to test for phase I and phase II.

Example 1

Hybrid Nanocurcumin Formulation: PLGA was dissolved in organic solvent, acetonitrile to get a concentration of 10 mg/ml. Curcumin (5%) was dissolved in this polymer-organic solvent phase. Lipids (DMPC and DMPG) were mixed in a different molar ratios and volume was made up to 1 ml. In more detail:

Hybrid Nanocurcumin Formulation: Polymer PLGA (10 mg) was dissolved in 1 ml of organic solvent, acetonitrile to get a concentration of 10 mg/ml. Curcumin (5% with respect to polymer) was dissolved in this polymer-organic solvent mixture. Lipids (DMPC and DMPG) were mixed in different molar ratios, and it was found that a ratio DMPC/DMPG=7.5/2.5 gave the best particles. DMPC (lipid 1) was dissolved in 4% ethanol in water. DMPG (lipid 2) was dissolved in water and volume was made up to 1 ml. These solutions were mixed and heated to obtained transparent solutions. Total lipid content with respect to polymer was varied from 2 mg to 8 mg. The organic phase was slowly stirred into the lipid aqueous phase keeping the organic to aqueous volume ratio at 1:1. The emulsion was vortexed for 30 sec and then sonicated for 5 min. The whole emulsion system was then stirred for 2-3 hours for self-assembly. This was then filtered thrice using Amicon filter (10 KD cutoff). The hybrid particles thus obtained were flash frozen using liquid nitrogen and lyophilized overnight. These were stored at −20° C. until further used.

The organic phase was slowly stirred into the lipid aqueous phase keeping the organic to aqueous volume ratio at 1:1. The emulsion was vortexed for 30 sec and then sonicated for 5 min. The whole emulsion system was then stirred for 2 hours for self-assembly. This was then filtered thrice using Amicon filter (10 KD cutoff). The hybrid particles thus obtained were flash frozen using liquid nitrogen and lyophilized overnight. These were stored at −20° C. until further used.

Hybrid Nanocurcumin Characterization: The hybrid nanoparticles were characterized for particle size, drug loading, encapsulation efficiency and surface morphology. FIG. **4**A shows results from one set of studies where the total amounts of lipids were varied keeping the molar ratio of two lipids constant. In other studies, lipids (DMPC and DMPG) were mixed in different molar ratios and we found that DMPC/DMPG::7.5/2.5 gave the best particles. In certain embodiments, the Hybrid Nanocurcumin is referred to herein as Curcumin ER.

Particle size distribution: The particle size distribution is shown in FIG. **4**B. The particle sizes for various batches post lyophilization are listed in Table 1. Particle size analysis of the lyophilized nanoparticles was carried out using a Nanotrac system (Mircotrac, Inc., Montgomeryville, Pa., USA). The lyophilized nanoparticles were dispersed in double distilled water and vortexed at high for 10 sec and then measured for particle size. The results were reported as the average of three runs with triplicate runs in each run.

TABLE 1

Average particle size distributions for all batches

Batch	DMPC + DMPG (mg)	Av. Particle Size (nm)
Batch 1	2	138.0
Batch 2	4	117.2
Batch 3	6	142.7
Batch 4	8	103.6

Drug loading and encapsulation efficiency: The hybrid nanocurcumin was dissolved in acetonitrile and drug loading and encapsulation efficiency was determined by spectrophotometry. Values are listed in Table 2. Lyophilized hybrid nanoparticles (5 mg) was dissolved in 2 ml acetonitrile to extract curcumin into acetonitrile for determining the encapsulation efficiency. The samples in acetonitrile were gently shaken on a shaker for 4 h at room temperature to completely extract out curcumin from the nanoparticles into acetonitrile. These solutions were centrifuged at 14,000 rpm (Centrifuge 5415D, Eppendorf AG, Hamburg, Germany) and supernatant was collected. Suspension (20 μl) was dissolved in ethanol (1 ml) and was used for the estimations. The curcumin concentrations were measured spectrophotometrically at 450 nm. A standard plot of curcumin (0-10 μg/ml) was prepared under identical conditions.

The encapsulation efficiency (EE) of PLGA-CURC was calculated using

$$\text{Encapsulation efficiency (\%)} = \frac{\text{Total drug content in nanoparticles}}{\text{Total drug amount}} \times 100$$

The percent drug loading was calculated by total amount of drug extracted from the hybrid nanoparticles to the known weight of nanoparticles

$$\text{Drug loading(\%)} = \frac{\text{Drug content}}{\text{Weight of nanoparticles}} \times 100$$

TABLE 2

Drug loading and encapsulation efficiency for all batches.

Batch	Drug Loading (%)	Encapsulation efficiency (%)
Batch 1	0.5	10
Batch 2	0.6	12
Batch 3	1.0	20
Batch 4	0.3	6

Surface morphology: Surface morphology of the HNC was determined by Transmission electron microscopy. The TEM scan is shown below in FIG. **3**. The surface morphology of the hybrid nanoparticle was studied using transmission electron microscopy, (TEM). A small quantity of aqueous solution of the lyophilized hybrid nanoparticles (1 mg/ml) was placed on a TEM grid surface with a filter paper (Whatman No. 1). One drop of 1% uranyl acetate was added to the surface of the carbon-coated grid. After 1 minute of incubation, excess fluid was removed and the grid surface was air dried at room temperature. It was then loaded into the transmission electron microscope (LEO EM910, Carl Zeiss SMT Inc, NY, USA) attached to a Gatan SC 1000 CCD camera. HNC are characterized, which included determination of average particle size, drug loading, and encapsulation efficiency and results are shown in FIG. **5**.

Hybrid Nanocurcumin Evaluation: Hybrid nanocurcumin was evaluated by intracellular uptake and MTT assays. This study shows robust uptake of HNC within 1 hour in pancreatic cancer cell, MiaPaCa cells as shown in FIG. **8**. Intracellular uptake of nanoparticle was determined in pancreatic, prostate and breast cancer cells using a Confocal Laser Scanning Microscope (CLSM). For these studies, cells were placed on a cover slip in a 6-well tissue culture plate and incubated at 37° C. until they reached sub-confluent levels. The cells were then exposed to 100 μg/ml concentrations of fluorescent nile red labeled hybrid nanoparticles. After 2 hrs of incubation, cells were viewed under the microscope.

MTT Assay: This assay was carried out in pancreatic cancer cell line, MiaPaCa. The IC_{50} for the HNC formulation was found to be at 22 μM concentration (FIG. **10**). To determine the effect of hybrid nanoparticles on cell growth, cell viability (MTT) assay was carried out in pancreatic prostate and breast cancer cell lines. The inhibition in cell growth was measured by the MTT assay. For this assay, ~2000 cells/well were plated in a 96-well plate and were treated with different μM concentrations of free drug and equivalent doses of drug-loaded hybrid nanoparticles. The assay was terminated after 48 and 72 hours and relative growth inhibition compared to control cells was measured. All studies were set up in triplicates and repeated twice for statistical analysis. Results were expressed as mean±S.D.

Results of western blot analysis of MiaPaCa cells treated with hybrid nanocurcumin (25 μM (micromolar)); untreated; blank nanoparticle; Curcumin (24 hrs); HNC (24 hrs) and; HNC (48 hrs) are provided in FIG. **9**.

Example II

Evaluation of the effects of Liposoma-PLGA curcumin on the human potassium channel using human embryonic kidney (HEK) 293 cells transfected with a human ether-a-gogo-related gene (hERG): The example deals with quantifying the in vitro effects of Liposoma-PLGA curcumin on the potassium-selective IKr current generated in normoxic conditions in stably transfected HEK 293 cells. The hERG assay is used to assess the potential of a compound to interfere with the rapidly activating delayed-rectifier potassium channel; and is based on current International Conference on Harmonisation (ICH) Harmonized Tripartite Guidelines [ICH S7a/b] and generally accepted procedures for the testing of pharmaceutical compounds.

Study outline: Test articles: Batch A, Batch B, Batch C, Batch D and Batch E. Test System: hERG-expressing HEK 293 transfected cell line. Test performed: Whole-cell patch-clamp current acquisition and analysis. Study Temperature: 35+/−2° C.

Application of test articles, positive control and vehicle: 5 minutes of exposure to each concentration in presence of closed circuit perfusion (2 mL/min). 5 minutes for washout period in presence of a flow-through perfusion (2 mL/min) in addition to a closed circuit perfusion (2 mL/min). The positive control (100 nM E-4031) was added to naive cells obtained from the same cell line and same passage for a period of 5 minutes in presence of a closed circuit perfusion (2 mL/min).

Cells were under continuous stimulation of the pulses protocol throughout the studies and cell currents were recorded after 5 minutes of exposure to each condition.

Original data acquisition design is shown in FIG. **11**.

Design for acquisition when testing the test articles or vehicle:

1 recording made in baseline condition

1 recording made in the presence of concentration 1, 2 or 3

1 recording made after washout (only after the concentration 3)

Design for acquisition when testing the positive control:

1 recording made in baseline condition

1 recording made in the presence of the positive control

n=number of responsive cells patched on which the whole protocol above could be applied

Statistical analysis: Statistical comparisons were made using paired Student's t-tests. For the test articles, the currents recorded after exposure to the different test article concentrations were statistically compared to the currents recorded in baseline conditions. Currents recorded after the washout were statistically compared to the currents measured after the highest concentration of test articles. In the same way, currents recorded after the positive control were compared to the currents recorded in baseline conditions.

Differences were considered significant when $p<0.05$.

Exclusion criteria:

1. Timeframe of drug exposure not respected
2. Instability of the seal
3. No tail current generated by the patched cell
4. No significant effect of the positive control
5. More than 10% variability in capacitance transient amplitude over the duration of the study.

Effect of the Test Articles on Whole-Cell IKr hERG Currents:

Whole-cell currents elicited during a voltage pulse were recorded in baseline conditions and following the application of the selected concentrations of test articles. Currents were also recorded following a washout period. The cells were depolarized for one second from the holding potential (−80 mV) to a maximum value of +40 mV, starting at −40 mV and progressing in 10 mV increments. The membrane potential was then repolarized to −55 mV for one second, and finally returned to −80 mV.

Whole-cell tail current amplitude was measured at a holding potential of −55 mV, following activation of the current from −40 to +40 mV. Current amplitude was measured at the maximum (peak) of this tail current. Current density was obtained by dividing current amplitude by cell capacitance measured prior to capacitive transient minimization. As per protocol, 3 concentrations of each test article were analyzed for hERG current inhibition.

Result of the studies showing hERG current density analysis of curcumin; liposomal curcumin; and PLGA curcumin are provided in FIGS. **6** and **7**, which show hERG current density analysis of liposomes+curcumin; and liposomes.

Current run-down and solvent effect correction. All data points presented in this Results Report have been corrected for solvent effect and time-dependent current run-down. Current run-down and solvent effects were measured simultaneously by applying the study design in test-article free conditions (hERG external solution or DMSO) over the same time frame as was done with the test articles. The loss in current amplitude measured during these so-called vehicle studies (representing both solvent effects and time-dependent run-down) was subtracted from the loss of amplitude measured in the presence of the test articles to isolate the effect of the test articles, apart from the effect of the solvent and the inevitable run-down in current amplitude over time.

This results, as shown in FIG. **11-27**, quantify the effect of Liposomal-PLGA curcumin (Batch A, Batch B, Batch C, Batch D and Batch E) on the rapidly activating delayed-rectifier potassium selective current (IKr) generated under normoxic conditions in stably transfected Human Embryonic Kidney (HEK) 293 cells.

The concentrations of curcumin (6, 12 and 18 μM) were selected and reflect a range estimated to exceed the therapeutic.

To confirm the reversal effect of the test articles, cells exposed to the highest concentration (18 μM) were subject to a washout period of 5 minutes. The current measured after the washout period was not statistically different when compared to the current left after highest concentration exposure of the compounds showing that the effect of these compounds was not reversible.

E-4031 is one of the most selective hERG inhibitors available to date. It was selected to demonstrate the sensitivity of the test system. Three naive HEK293-hERG cells were exposed to 100 nM E-4031. E-4031 induced a significant inhibition of 81.8% of the current amplitude for I+20.

Sample Information: Store at −20° C., and protected from direct sunlight:

1) Batch A—
   Total weight of sample—215 mg
   Curcumin content—18 micro g/mg of test sample
   Material used—Polymer (PLGA), Lipid (DMPC+DMPG), Curcumin, sucrose.
2) Batch B—
   Total weight of sample—200 mg
   Curcumin content—6.8 micro g/mg of test sample
   Material used—Polymer (PLGA), Lipid (DMPC+DMPG), Curcumin, sucrose.
3) Batch C—
   Total weight of sample—200 mg
   Curcumin content—18.2 micro g/mg of test sample
   Material used—Polymer (PLGA), Chitosan, Polyvinyl alcohol (PVA), Lipid (DMPC+DMPG), Curcumin, sucrose.
4) Batch D—Pure curcumin
   Total weight—50 mg.
5) Batch E—Liposomal curcumin
   Total volume—5 ml
   Curcumin content—6.4 mg/ml
   Material used—Lipid (DMPC+DMPG), Curcumin
   Molecular weight information:
   Curcumin Molecular weight—368.38 g/mol
   PLGA (50:50)—Molecular weight—124 kDa
   DMPC (PC (14:0/14:0))—Molecular weight—677.933 g/mol
   DMPG—Molecular weight—688.845 g/mol
   Sucrose—Molecular Weight 342.30 g/mol
   Chitosan—Low Molecular weight—75-85% deacetylated
   Polyvinyl alcohol (PVA)—Average molecular weight—30,000-70,000.

It is contemplated that any embodiment discussed in this specification can be implemented with respect to any method, kit, reagent, or composition of the invention, and vice versa. Furthermore, compositions of the invention can be used to achieve methods of the invention.

Evaluation of the Effects of Curcumin ER and Liposomal Curcumin on H-460 and A-549 Lung Cancer Mouse Xenograft Model.

The purpose of this study was to quantify the mean tumor volume of the mouse xenograft model over duration of the treatment. Specifically, the encapsulated and liposomally coated Curcumin ER and Liposomal Curcumin were tested using the cell lines H-460 and A-549, lung cancer xenograft model. Briefly, Female Hsd:athymic Nude-Foxn1nu mice 3-4 weeks old were obtained from Harlan Laboratories, USA. The cancer cells were injected into the mice and tumor volume was evaluated. The liposomal curcumins, Curcumin ER and Liposomal Curcumin, were administered via subcutaneous injection at a dose of 20 mg/kg body weight once in a week.

FIG. **28** shows the results of the treatment of the H-460 breast cancer cell line in the Hsd:Athymic Nude-Foxn1nu mice. FIG. **29** shows additional results of the treatment of the A-549 breast cancer cell line in the Hsd:athymic Nude-Foxn1nu mice.

It will be understood that particular embodiments described herein are shown by way of illustration and not as limitations of the invention. The principal features of this invention can be employed in various embodiments without departing from the scope of the invention. Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, numerous equivalents to the specific procedures described herein. Such equivalents are considered to be within the scope of this invention and are covered by the claims.

All publications and patent applications mentioned in the specification are indicative of the level of skill of those skilled in the art to which this invention pertains. All publications and patent applications are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

The use of the word “a” or “an” when used in conjunction with the term “comprising” in the claims and/or the specification may mean “one,” but it is also consistent with the meaning of “one or more,” “at least one,” and “one or more than one.” The use of the term “or” in the claims is used to mean “and/or” unless explicitly indicated to refer to alternatives only or the alternatives are mutually exclusive, although the disclosure supports a definition that refers to only alternatives and “and/or.” Throughout this application, the term “about” is used to indicate that a value includes the inherent variation of error for the device, the method being employed to determine the value, or the variation that exists among the study subjects.

As used in this specification and claim(s), the words “comprising” (and any form of comprising, such as “comprise” and “comprises”), “having” (and any form of having, such as “have” and “has”), “including” (and any form of including, such as “includes” and “include”) or “containing” (and any form of containing, such as “contains” and “contain”) are inclusive or open-ended and do not exclude additional, unrecited elements or method steps.

The term “or combinations thereof” as used herein refers to all permutations and combinations of the listed items preceding the term. For example, “A, B, C, or combinations thereof” is intended to include at least one of: A, B, C, AB, AC, BC, or ABC, and if order is important in a particular context, also BA, CA, CB, CBA, BCA, ACB, BAC, or CAB. Continuing with this example, expressly included are combinations that contain repeats of one or more item or term, such as BB, AAA, AB, BBC, AAABCCCC, CBBAAA, CABABB, and so forth. The skilled artisan will understand that typically there is no limit on the number of items or terms in any combination, unless otherwise apparent from the context. In certain embodiments, the present invention may also include methods and compositions in which the transition phrase “consisting essentially of” or “consisting of” may also be used.

All of the compositions and/or methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of this invention have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the compositions and/or methods and in the steps or in the sequence of steps of the method described herein without departing from the concept, spirit and scope of the invention. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.

What is claimed is:

1. A method of forming a nanoparticle that does not cause QT prolongation when provided to a subject composition comprising:

forming an organic phase by combining one or more polymers, one or more solvents and at least one of curcumin or curcuminoids;

forming a lipid aqueous phase by mixing one or more lipids with water;

mixing the organic phase with the lipid aqueous phase, whereby an emulsion is formed; and

incubating the emulsion, whereby self-assembly of nanoparticles occurs and wherein the curcumin or curcuminoids nanoparticles does not cause QT prolongation when provided to a subject.

2. The method of claim **1**, wherein the one or more polymers comprise at least one or poly(lactic-co-glycolic acid) (PLGA), poly(lactic acid), polylactide (PLA), and poly-L-lactide-co-ϵ-caprolactone (PLCL).

3. The method of claim **1**, wherein the organic phase comprises PLGA in a concentration of 2-90 mg/ml.

4. The method of claim **1**, wherein the organic phase comprises curcumin in a concentration of 1-15 weight % to volume.

5. The method of claim **1**, wherein the one or more solvents comprises an organic solvent selected from at least one or acetonitrile, acetone, tert butyl alcohol, dimethyl formamide, and hexafluro isopropanol.

6. The method of claim **1**, wherein the one or more lipids comprise at least one or DMPC, DMPG, 1,2-dioctadecanoyl-sn-glycero-3-phosphoethanolamine (DSPE), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[amino(polyethylene glycol) (DSPE-PEG), DMPE PEG Maleimide, Lecithin, cholesterol, 1,2-dimyristoyl-sn-glycero-3-phosphoethanolamine-N-(lissamine rhodamine B sulfonyl) (ammonium salt), and 1,2-dimyristoyl-sn-glycero-3-phosphoethanolamine-N-(7-nitro-2-1,3-benzoxadiazol-4-yl) (ammonium salt).

7. The method of claim **1**, wherein the one or more lipids comprise DMPC and DMPG in a molar ratio of 9:1, 7:3, 8:2, or 7.5:2.5.

8. The method of claim **1**, wherein mixing the organic phase with the lipid aqueous phase comprises at least one of stirring the organic phase into the lipid aqueous phase, mixing the organic phase with the lipid aqueous phase comprises vortexing, or mixing the organic phase with the lipid aqueous phase further comprises sonicating.

9. The method of claim **1**, wherein incubating the emulsion comprises stirring the emulsion for 2 hours.

10. The method of claim **1**, further comprising one or more of the following: (1) separating the nanoparticles after incubating the emulsion; (2) filtering the nanoparticles after incubating the emulsion; (3) freezing the nanoparticles; (4) lyophilizing the nanoparticles; or (5) attaching a targeting agent to the nanoparticles.

11. The method of claim **1**, further comprising the attaching at least one targeting agent, wherein the targeting agent selectively targets the nanoparticle to diseased tissue/cells, thereby minimizing whole body dose.

12. The method of claim **1**, further comprising attaching at least one targeting agent to the nanoparticles, wherein the targeting agent comprises an antibody or functional fragment thereof that is capable of recognizing a target antigen.

13. The method of claim **1**, wherein the nanoparticles have a size of 90 to 150 nm.

14. A method of forming a nanoparticle that prevents the active agent from causing QT prolongation caused by curcumin or a curcuminoid comprising:

forming an organic phase by combining one or more polymers, one or more solvents and the active agent that causes QT prolongation;

forming a lipid aqueous phase by mixing one or more lipids with water;

mixing the organic phase with the lipid aqueous phase, whereby an emulsion is formed; and

incubating the emulsion, whereby self-assembly of nanoparticles occurs, wherein the nanoparticle does not cause QT prolongation when provided to a subject.

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